



The southernmost distribution of the Eastern Black-bridged Leaf Turtle, *Cyclemys pulchristriata* Fritz, Gaulke & Lehr, 1997 (Reptilia, Testudines, Geoemydidae), in Ba Ria–Vung Tau Province, Vietnam

Luan Thanh Nguyen¹, Vu Dang Hoang Nguyen², Sang Ngoc Nguyen²

1 Asian Turtle Program - Indo-Myanmar Conservation, R.1806, 18th F., CT1 Bac Ha C14 Building To Huu St., Nam Tu Liem District, Ha Noi, Vietnam; nguyenthanhluan@asianturtleprogram.org. **2** Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan St., Dist. 3, Ho Chi Minh City, Vietnam.

Corresponding author: Sang Ngoc Nguyen, e-mail: ngocsangitb@yahoo.com

Abstract

We report the southernmost occurrence of the Eastern Black-bridged Leaf Turtle, *Cyclemys pulchristriata* Fritz, Gaulke & Lehr, 1997, from an isolated mountain in Ba Ria–Vung Tau Province, southern Vietnam, based on morphological and molecular data of a juvenile captured in the wild. This record extends the distribution of this species approximately 150 km south of previous records and 600 km from its type locality in Quang Nam Province, Central Vietnam.

Key words

Freshwater turtle; Mount Dinh; range extension; Vietnam.

Academic editor: Ross MacCulloch | Received 21 August 2018 | Accepted 18 October 2018 | Published 9 November 2018

Citation: Nguyen LT, Nguyen VD, Nguyen SN (2018) The southernmost distribution of the Eastern Black-bridged Leaf Turtle, *Cyclemys pulchristriata* Fritz, Gaulke & Lehr, 1997 (Reptilia, Testudines, Geoemydidae), in Ba Ria–Vung Tau Province, Vietnam. Check List 14 (6): 1007–1011. <https://doi.org/10.15560/14.6.1007>

Introduction

The genus *Cyclemys* Bell, 1834 comprises 7 species distributed throughout South and Southeast Asia (Fritz et al. 2008, Turtle Taxonomy Working Group 2017). Two species, *Cyclemys oldhamii* Gray, 1863 and *C. pulchristriata* Fritz, Gaulke & Lehr, 1997, are known to occur in Vietnam (Turtle Taxonomy Working Group 2017). The Eastern Black-bridged Leaf Turtle, *Cyclemys pulchristriata*, was originally described from Central Vietnam (Phuc Son, Quang Nam Province) and subsequently recorded from Lam Dong, Phu Yen, Gia Lai, and Binh Dinh provinces in Vietnam and Mondolkiri Province in Cambodia (Fritz et al. 1997, Fritz et al. 2008, Nguyen et al. 2009). The most similar species of *C. pulchristriata* is *C. atripons* Iverson & McCord, 1997 which occurs

in south-eastern Thailand and south-western Cambodia. Morphologically, both species are similar to each other but they can be distinguished conclusively from each other by DNA sequences (Fritz et al. 2008). In a recent field survey in southern Vietnam, we captured a single yellow-bellied juvenile turtle that exhibits morphological characteristics of *C. atripons* and *C. pulchristriata*. Molecular analysis confirms that the specimen belongs to *C. pulchristriata*. Thus, we provide a new southernmost record of this species in Vietnam.

Methods

Sampling. Field work was conducted on 3 March 2018 in Mount Dinh, Tan Thanh District, Ba Ria–Vung Tau Province, southern Vietnam. A specimen was captured,

photographed, and measured. A tissue sample for molecular analysis was taken from its tail tip, stored in absolute alcohol and subsequently deposited in the Institute of Tropical Biology Zoological Collection (ITBCZ), Ho Chi Minh City, Vietnam (ITBCZ 6011). The turtle was released at its point of capture immediately after handling. A total of 29 photographs were taken and deposited in the ITBCZ under numbers ITBCZ 6011-1–ITBCZ 6011-29. Under Vietnamese regulations, no permit was required for this procedure.

Laboratory procedures. DNA extraction, amplification, and sequencing of the mitochondrial *cyt b* gene followed Fritz et al. (2008). The GenBank accession number of the sequence is MK014303 (Table 1).

Phylogenetic analyses. The dataset for phylogenetic analyses of the mitochondrial *cyt b* gene (984 bp) was selected from Fritz et al. (2008), plus our new sequence. A total of 26 sequences were chosen. The dataset included all available sequences of *C. pulchristriata*, 1 sequence of *C. gemeli* Fritz, Guicking, Auer, Sommer, Wink & Hundsdörfer, 2008, 2 sequences of each remaining species of *Cyclemys*, and the sequence of our turtle (Table 1). *Heosemys spinosa* (Gray, 1830) was chosen as outgroup taxon based on Fritz et al. (2008). Among the 13 available sequences of *Cyclemys pulchristriata*, only 1 sequence originates from a wild-caught specimen, in Cambodia (AM931681, Fig. 1), the others come from either trade or market. Hence, ours is the first sequence

originating from the wild in Vietnam.

The raw nucleotide sequence generated was initially verified by eye using SEQMAN PRO™ (DNASTAR Lasergene 7, Madison, WI). Selected sequences were aligned using the CLUSTALW (Thompson et al. 1997) option in MEGA 7.0 (Kumar et al. 2016) with default parameters. Mean uncorrected genetic distances (*p*-distances) between sequences and species were calculated using MEGA 7.0. MODELTEST v.3.06 (Posada and Crandall 1998) was used to estimate the optimal evolutionary models for the dataset. The best-fit model was the GTR+I model of DNA evolution as suggested by the Akaike Information Criterion.

The matrilineal genealogy was inferred using Bayesian inference (BI) and Maximum Likelihood (ML). BI was conducted in MRBAYES v.3.1.2 (Ronquist and Huelsenbeck 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with 1 cold chain and 3 heated chains for 4 million generations and sampled every 1000 generations. Five independent MCMCMC runs were performed and 1000 trees were discarded as burn-in. Confidence in topology was assessed by posterior probability (BPP, Huelsenbeck and Ronquist 2001). The ML analysis was conducted using TREEFINDER (Jobb et al. 2004) and confidence in node topology was tested by non-parametric bootstrapping with 1000 replicates (BS, Felsenstein 1985).

Morphology. Five measurements were taken using a caliper to nearest 0.1 mm: carapace length, carapace width,

Table 1. *Cyclemys* sequences of *Cyt b* gene used in this study. Dataset obtained from Fritz et al. (2008) and a new sequence from this study; see Fritz et al. (2008) for museum abbreviations.

Species	Locality	Sample	GenBank no.	Remarks/reference
<i>atriponts</i>	Thailand: Trat Province: Kao Kuap	USNM 81865	DQ444271	Holotype of <i>C. atriponts</i>
<i>atriponts</i>	Thailand: Trat Province: Koh Chang	USNM 53423	DQ444270	Paratype of <i>C. atriponts</i>
<i>dentata</i>	Indonesia	MTD T 4210	AM931635	International pet-trade
<i>dentata</i>	Indonesia: Java	BMNH 1946.1.22.62	DQ444272	Lectotype of <i>C. dentata</i>
<i>enigmatica</i>	Indonesia: Sumatra: Padang	NHMW 9811	AM931644	Holotype of <i>C. enigmatica</i>
<i>enigmatica</i>	Malaysia: Penang	MTD T 4226	AM931645	Obtained from local turtle-trader
<i>fusca</i>	Myanmar: Kachin State	MTD D 42578	AM931651	Holotype of <i>C. fusca</i>
<i>fusca</i>	Myanmar: Sagaing Division	CAS 210070	AM931654	Field-collected
<i>gemeli</i>	India: Assam: Jia Bhoroli River Region	NHMW 37153	AM931656	Holotype of <i>C. gemeli</i>
<i>oldhamii</i>	Laotian/Vietnamese border region: vicinity of Lao Bao	MTD D 42537	AM931666	Obtained from local turtle-trader
<i>oldhamii</i>	Myanmar: Tanintharyi: Mergui	BMNH 1947.3.5.63	DQ444274	Lectotype of <i>C. oldhamii</i>
<i>pulchristriata</i>	Cambodia: Mondolkiri	FMNH 259050	AM931681	Field-collected
<i>pulchristriata</i>	Unknown	MTD D 42549	AM931682	Cambodian turtle-trade
<i>pulchristriata</i>	Unknown	MTD D 42550	AM931683	Cambodian turtle-trade
<i>pulchristriata</i>	Unknown	MTD D 42551	AM931684	Cambodian turtle-trade
<i>pulchristriata</i>	Unknown	MTD D 42552	AM931685	Cambodian turtle-trade
<i>pulchristriata</i>	Unknown	MTD D 43785	AM931686	Obtained at Cau-Mong market, Saigon
<i>pulchristriata</i>	Unknown	MTD D 44261	AM931687	International pet-trade
<i>pulchristriata</i>	Unknown	MTD D 44262	AM931688	International pet-trade
<i>pulchristriata</i>	Unknown	MTD T 18	AM931689	Obtained at Cau-Mong market, Saigon
<i>pulchristriata</i>	Unknown	MTD T 19	AM931690	Obtained at Cau-Mong market, Saigon
<i>pulchristriata</i>	Unknown	MTD T 20	AM931691	Obtained at Cau-Mong market, Saigon
<i>pulchristriata</i>	Unknown	MTD T 4206	AM931692	Obtained at Cau-Mong market, Saigon
<i>pulchristriata</i>	Unknown	MTD T 4209	AM931693	Obtained at Cau-Mong market, Saigon
<i>pulchristriata</i>	Unknown	—	AY434617	Pet trade
<i>pulchristriata</i>	Vietnam: Ba Ria-Vung Tau	ITBCZ 6011	MK014303	Field-collected

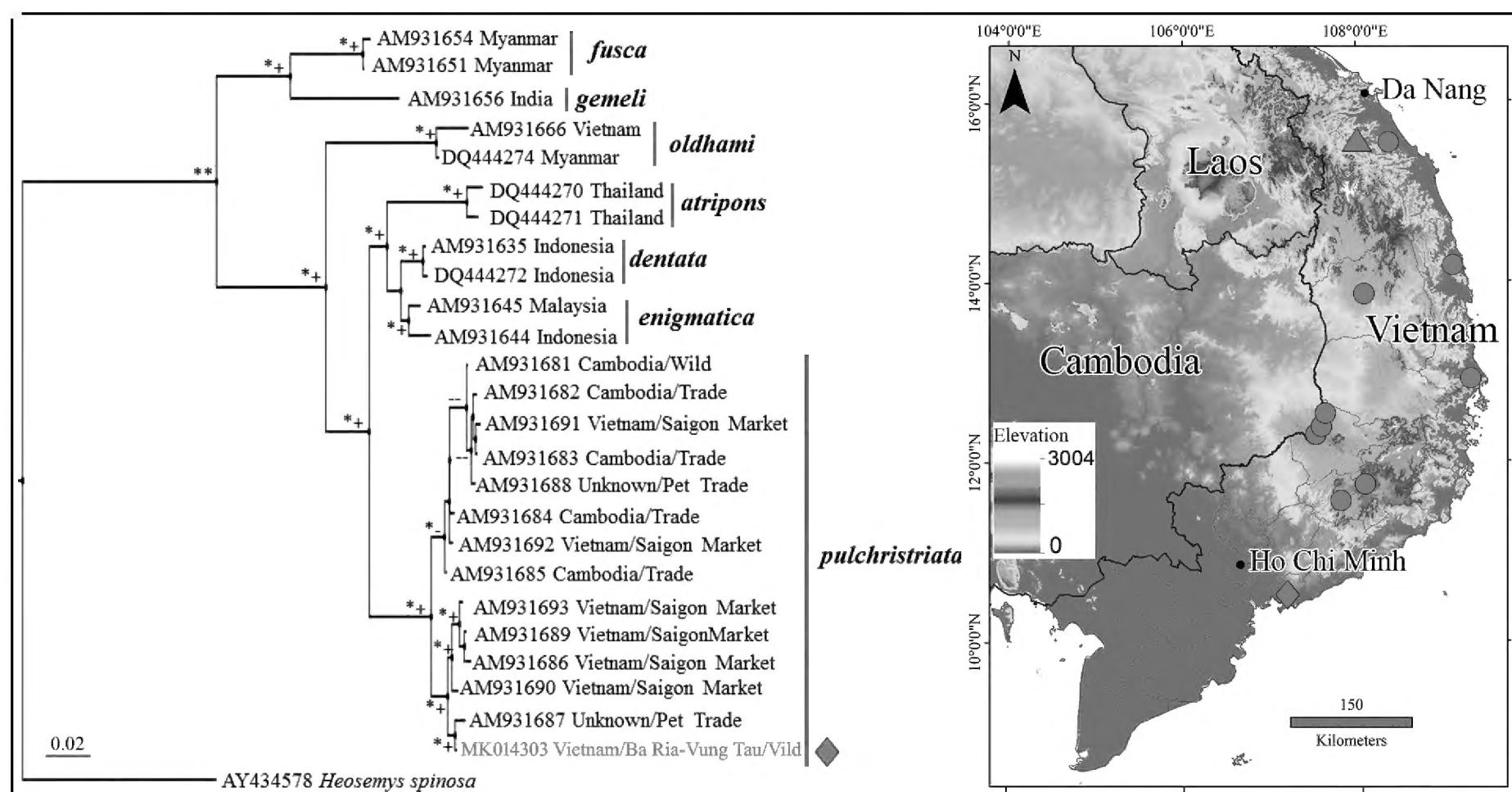


Figure 1. The Bayesian inference (BI) phylogeny for *Cyclemys* (left) and distribution of *C. pulchristriata* (right). The type locality is marked with a pink triangle, previous records are marked by pink circles, and new record is marked by a blue diamond. Values at nodes are BPP/BS support values, respectively. Asterisk: supported 100% by BI and ML; plus sign: supported 70–99% by ML; hyphen: unsupported.

plastron length (maximum), plastron length (mid-seam), and tail length.

Results

New record. Vietnam, Ba Ria–Vung Tau Province, Tan Thanh District, Mount Dinh ($10^{\circ}32'21''$ N, $107^{\circ}08'06''$ E), ca 420 m above sea level (Fig. 1), observed by S.N. Nguyen & V.D.H. Nguyen, 3 March 2018, 1 juvenile of unknown sex, found in a small stream in evergreen forest.

Species description. Morphological characteristics of our specimen match with the description of species *Cyclemys pulchristriata* (Fritz et al. 1997, Fritz et al. 2008). The specimen is a juvenile, carapace length 91 mm, width 75 mm; oval in dorsal view and low in lateral view; rear carapace margin distinctly serrated. Plastron oval, maximum length 72 mm, mid-seam length 70 mm; hyo-hypoplastral hinge present. Tail narrow, 23 mm in length. Head blackish brown above, yellow with 2 black stripes laterally; neck and throat bright yellow. Carapace yellowish brown, black radiating lines present on each scute. Plastron and ventral marginals yellow; plastral scutes with black radiating lines, broader than on capacious scutes (Fig. 2).

Phylogeny, genetic distance, and taxonomic remarks. Our trees are similar to those of Fritz et al. (2008). Both BI and ML analyses showed that our sample from Mount Dinh is embedded in the branch of *C. pulchristriata* with high supports (BPP and BS = 1.0 and 99%, respectively), suggesting that our sample is *C. pulchristriata*. Although juveniles of *Cyclemys pulchristriata* closely resemble *C. atripons* (Fritz et al. 2008), genetic analyses confirm that our sample is *C. pulchristriata*. The uncorrected

p-distance between our sample from Mount Dinh and all available sequences of *C. pulchristriata* ranges from 0.00% to 1.80% (ingroup mean uncorrected *p*-distances of *C. pulchristriata* sequences including our sample is 0.80%). The *p*-distance between *C. pulchristriata* and *C. atripons* is 6.26% and between *C. pulchristriata* and other remain species of *Cyclemys* range from 5.22% (*C. dentata*) to 13.5% (*C. fusca*).

Discussion

The previous southernmost distribution of this species is in Lam Dong Province, Vietnam (Fritz et al. 2008, Nguyen et al. 2009). Recent studies have filled the gap of distribution of this species in Binh Dinh and Phu Yen provinces, Central Vietnam (Duong et al. 2014, Do et al. 2016). The observation of this species in Ba Ria–Vung Tau extends the distribution of this species about 150 km south from the previous record in Bao Loc District, Lam Dong Province and 600 km from its type locality in Quang Nam Province. In addition, our new record of *Cyclemys pulchristriata* in Vietnam is approximately 350 km from the closest record of *C. atripons* in south-western Cambodia. Further research in southern Vietnam is needed to fully understand the distribution of this species.

Acknowledgements

We would like to thank Tim McCormack of the Asian Turtle Program for confirming species identification. This work was funded by the Institute of Tropical Biology (official dispatch No. 20/QĐ-SHND of 2 February 2018) to SNN.

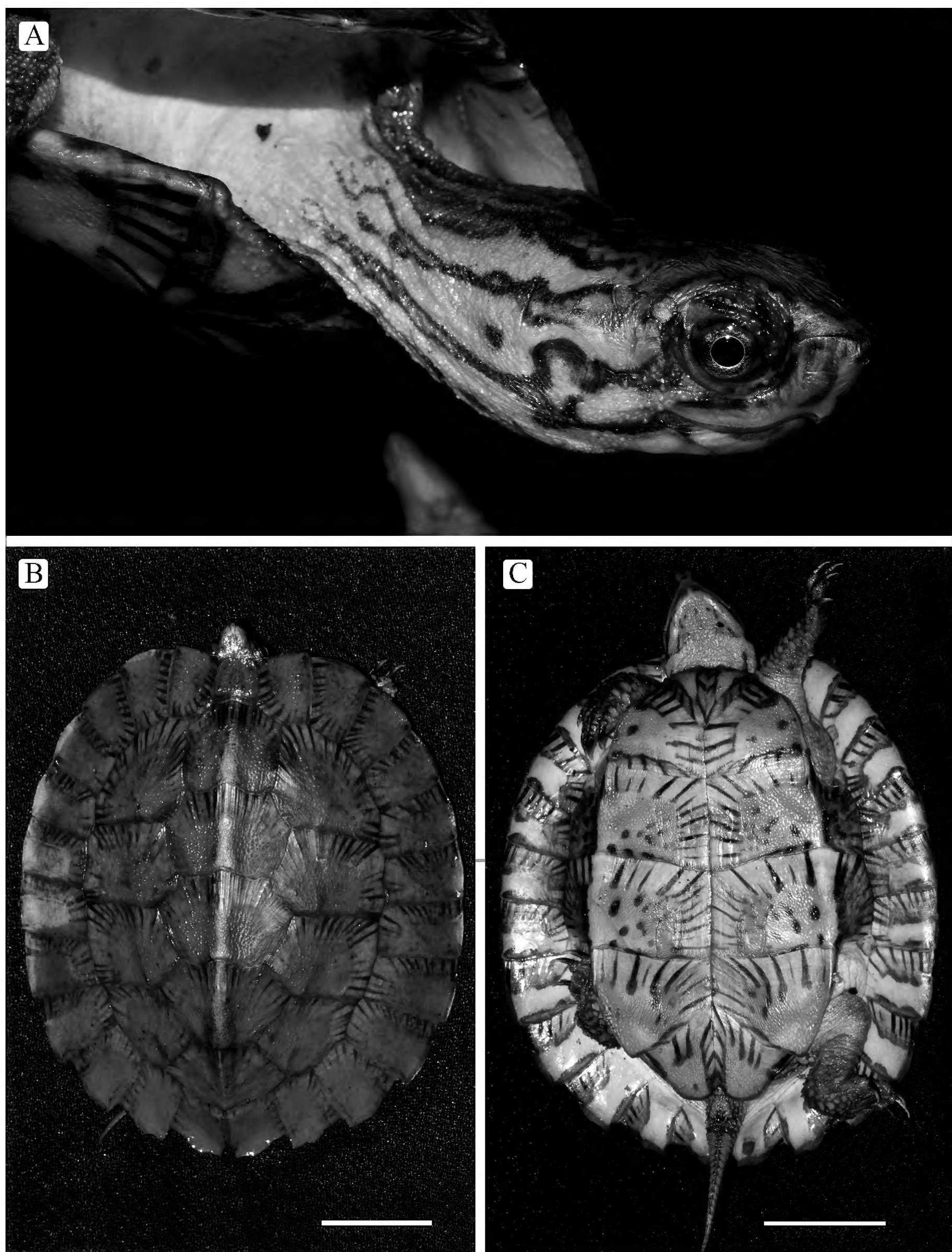


Figure 2. *Cyclemys pulchristriata* from Mount Dinh, Ba Ria-Vung Tau Province. **A.** lateral view of head. **B.** View of carapace. **C.** View of plastron. Scale bars = 2 cm.

Authors' Contributions

LTN identified the specimen, analysed data, and wrote the manuscript; VDHN collected material in the field; SNN collected material in the field and reviewed the manuscript.

References

Do TD, Ngo DC, Nguyen QT (2016) New records of turtles in Phu Yen Province. The 3rd National Scientific Conference on Biological Research and Teaching in Vietnam. Da Nang University Publishing House, Quy Nhon, 129–136 pp. [in Vietnamese].
Duong LD, Ngo CD, Nguyen TQ (2014) New records of turtles from

Binh Dinh Province, Vietnam. Herpetology Notes 7: 737–744.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Fritz U, Gaulke M, Lehr E (1997) Revision of the Southeast Asian leaf turtle genus *Cyclemys* Bell, 1834, with description of a new species. Salamandra 33: 183–212.

Fritz U, Guicking D, Auer M, Sommer R, Wink M, Hundsdörfer A (2008) Diversity of the Southeast Asian leaf turtle genus *Cyclemys*: how many leaves on its tree of life? Zoologica Scripta 37 (4): 367–390. <https://doi.org/10.1111/j.1463-6409.2008.00332.x>

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17 (8): 754–755.

Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evolutionary Biology 4: 18. <https://doi.org/10.1186/1471-2148-4-18>

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33 (7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>

Nguyen SV, Ho CT, Nguyen TQ (2009) Herpetofauna of Vietnam. Edition chimaira, Frankfurt am Main, 768 pp.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9): 817–818. <https://doi.org/10.1093/bioinformatics/14.9.817>

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19 (12): 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research 25: 4876–4882. <https://doi.org/10.1093/nar/25.24.4876>

Turtle Taxonomy Working Group [Rhodin AGJ, Inverson JB, Bour R, Fritz U, Geogres A, Shaffer HB, Van Dijk PP] (2017) Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status (8th ed.). Chelonian Research Monographs 7: 1–292. <https://doi.org/10.3854/crm.7.checklist.atlas.v8.2017>